## CLAIMS

1. A transposon which comprises an RNA polymerase recognition site and a homing endonuclease recognition site.

2. A transposon according to claim 1 which comprises two RNA polymerase recognition sites.

3. A transposon according to claim 2, wherein the two RNA polymerase recognition sites are diverse.

4. A transposon according to claim 3, wherein the two diverse RNA polymerase recognition sites are two of a T7 RNA polymerase recognition site, an SP6 RNA polymerase recognition site or a T3 RNA polymerase recognition site.

5. A transposon according to any one of the preceding claims which comprises two homing endonuclease recognition sites.

6. A transposon according to claim 5, wherein the two homing endonuclease recognition sites are diverse.

7. A transposon according to claim 6, wherein the two diverse homing endonuclease recognition sites are an I-*Sce*I recognition site and a PI-*Psp*I recognition site.

8. A transposon according to any one of the preceding claims which further comprises a bacterial origin of replication.

9. A transposon according to any one of the preceding claims which is a modified *Tn*5 transposon.

10. A transposon according to any one of claims 1 to 8 which is a modified *Mariner* transposon.

11. Use of a transposon according to any one of the preceding claims in a method for the identification of an essential or a conditional essential gene.

12. A method for identifying an essential gene of an organism, which method comprises:

(i) providing a library of transposon insertion mutants of the said organism, wherein the transposon is a transposon according to any one of claims 1 to 10;

(ii) isolating chromosomal DNA from the library of (i);

(iii) digesting the chromosomal DNA with a restriction endonuclease that is capable of cutting 5' to the RNA polymerase recognition site(s) in the transposon and 3' to the RNA polymerase recognition site(s) in the chromosomal DNA flanking the transposon insertion site;

(iv) transcribing the resulting digested DNA from the RNA polymerase recognition site(s) in the said DNA;

(v) hybridising the resulting RNA with an oligonucleotide array; and

(vi) identifying a probe on the oligonucleotide array which corresponds to an essential gene of the organism.

13. A method according to claim 12, wherein a labelled ribonucleotide is present when transcribing the digested DNA in step (iv).

14. A method according to claim 12, wherein step (v) is replaced by:

(v)' reverse transcribing the resulting RNA; and

(v)" hybridising the resulting cDNA with an oligonucleotide array.

15. A method according to claim 14, wherein a labelled deoxyribonucleotide is present when reverse transcribing the RNA in step (v)'.

16. A method according to claim 12 or 13, wherein:

(a) the transposon is a transposon according to any one of claims 3 to 10;

(b) step (iv) is carried out by transcribing one aliquot of the digested DNA with a first RNA polymerase and transcribing a second aliquot of the digested DNA with a second different RNA polymerase; and

(c) step (v) is carried out by hybridising the two resulting RNA pools with the same oligonucleotide array or separately with two copies of the same oligonucleotide array.

17. A method according to claim 16, wherein in step (b) the two aliquots of digested DNA are each transcribed in the presence of a different labelled ribonucleotide.

18. A method according to claim 14 or 15, wherein:

(a) the transposon is a transposon according to any one of claims 3 to 10;

(b) step (iv) is carried out by transcribing one aliquot of the digested DNA with a first RNA polymerase and transcribing a second aliquot of the digested DNA with a second different RNA polymerase; and

(c) step (v)" is carried out by hybridising the two resulting cDNA pools with the same oligonucleotide array or separately with two copies of the same oligonucleotide array.

19. A method according to claim 18, wherein the two aliquots of RNA resulting from step (b) are each reverse transcribed using a different labelled deoxyribonucleotide.

20. A method according to any one of claims 12 to 19, wherein:

(a) aliquots of the chromosomal DNA are digested separately with different restriction endonucleases in step (iii);

(b) each of the restriction endonucleases is capable of cutting 5' to the RNA polymerase recognition site(s) in the transposon and 3' to the RNA polymerase recognition site(s) in the chromosomal DNA flanking the transposon insertion site; and

(c) each aliquot is subsequently treated separately in steps (iv) to (vi).

21. A method according to claim 20, wherein two or three aliquots of the chromosomal DNA are each digested separately with different restriction endonucleases.

22. A method according to any one of claims 12 to 21, wherein step (iii) is replaced by:

(iii)' digesting the chromosomal DNA with a homing endonuclease which is capable of cutting 5' to RNA polymerase recognition site(s) in the transposon;

(iii)" digesting the chromosomal DNA with a restriction endonuclease that is capable of cutting 3' to the RNA polymerase recognition site(s) in the chromosomal DNA flanking the transposon insertion site; and

(iii)'" ligating the digested DNA with a biotinylated linker; and

(iii)'"' recovering the digested DNA using streptavidin-coated particles.

23. A method for identifying a conditional essential gene of an organism, which method comprises:

(a) providing a first sample of a library of transposon insertion mutants of the said organism (input library);

(b) providing a second sample of the library and subjecting that sample to a conditional restraint;

(c) collecting the mutants that survive the conditional restraint in step (ii)

to give a second library (output library);

(d) carrying out a method according to steps (ii) to (iv) of any one of claims 12, 13, 16, 17 or 20 to 22 on the input library from step (a) and on the output library from step (c);

(e) hybridising the transcribed RNA derived from the input library and from the output library separately to copies of the same oligonucleotide array or, if the RNA derived from the two libraries is differentially labelled, to the same oligonucleotide array; and

(f) identifying a probe on the oligonucleotide array(s) which corresponds to a conditional essential gene of the organism.

24. A method for identifying a conditional essential gene of an organism, which method comprises:

(a) providing a first sample of a library of transposon insertion mutants of the said organism (input library);

(b) providing a second sample of the library and subjecting that sample to a conditional restraint;

(c) collecting the mutants that survive the conditional restraint in step (ii) to give a second library (output library);

(d) carrying out a method according to steps (ii) to (v)' of any one of claims 14, 15, 18, 19 or 20 to 22 on the input library from step (a) and on the output library from step (c);

(e) hybridising the reverse transcribed cDNA derived from the input library and from the output library separately to copies of the same oligonucleotide array or, if the cDNA derived from the two libraries is differentially labelled, to the same oligonucleotide array; and

(f) identifying a probe on the oligonucleotide array(s) which corresponds to a conditional essential gene of the organism.

25. A method according to claim 23 or 24, wherein the organism is a bacterium and the conditional restraint is growth of that bacterium in its host.

26. A method according to any one of claims 12 to 25, wherein the oligonucleotide array comprises probes which are from 9 to 150 bp in length and/or comprises 1 probe for every 60 to 250 bp of the locus or loci represented on the array.

27. Use of an essential or conditional essential gene identified by a method according to any one of claims 12 to 26, or a polypeptide encoded by a said gene, in a method for identifying an inhibitor of transcription and/or translation of that gene and/or activity of a polypeptide encoded that gene.

28. A method for identifying an inhibitor of transcription and/or translation of an essential or conditional essential gene and/or an inhibitor of activity of a polypeptide encoded by a said gene, which method comprises:

(a) identifying an essential or conditional essential gene by a method according to any one of claims 12 to 26; and

(b) determining whether a test substance can inhibit transcription and/or translation of a gene identified in step (a) and/or activity of a polypeptide encoded by a said identified gene, thereby to identify a said inhibitor.

29. An inhibitor identified by a method according to claim 28.

30. An inhibitor according to claim 29 for use in a method of treatment of a bacterial, fungal or eukaryotic parasite infection, wherein the essential or conditional essential gene in claim 28 is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene.

31. Use of an inhibitor according to claim 29 wherein the essential or conditional essential gene in claim 28 is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene, in the manufacture of a medicament for use in the treatment of a bacterial, fungal or eukaryotic parasite infection.

32. A pharmaceutical composition comprising an inhibitor according to claim 29 wherein the essential or conditional essential gene in claim 28 is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene and a pharmaceutically acceptable carrier or diluent.

33. A method of treating a host suffering from a bacterial, fungal or eukaryotic parasite infection, which method comprises the step of administering to the host a therapeutically effective amount of an inhibitor according to claim 29 wherein the essential or conditional essential gene in claim 28 is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene.

34. A method for the preparation of a pharmaceutical composition, which method comprises:

(a) identifying an inhibitor of transcription and/or translation of an essential or conditional essential gene of an organism and/or an inhibitor of activity of a polypeptide encoded by a said gene by a method according to claim 28 wherein the essential or conditional essential gene is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene; and

(b) formulating the inhibitor thus identified with a pharmaceutically acceptable carrier or diluent.

35. A method for treating a host suffering from a bacterial, fungal or eukaryotic parasite infection, which method comprises:

(a) identifying an inhibitor of transcription and/or translation of an essential or conditional essential gene of an organism and/or an inhibitor of activity

of a polypeptide encoded by a said gene by a method according to claim 28 wherein the essential or conditional essential gene is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene;

(b) formulating the inhibitor thus identified with a pharmaceutically acceptable carrier or diluent; and

(c) administering to the host a therapeutically effective amount of an inhibitor thus formulated.

36. An inhibitor according to claim 29, wherein the essential or conditional essential gene in claim 28 is a plant bacterial, plant fungal or plant pest essential or conditional essential gene.

37. Use of an inhibitor according to 36 as a plant bactericide, fungicide or pesticide.

38. An inhibitor according to claim 29, wherein essential or conditional essential gene in claim 28 is a plant essential or conditional essential gene.

39. Use of an inhibitor according to claim 38 as a herbicide.

40. A bacterium attenuated by a non-reverting mutation in one or more genes identified by a method according to any one of claims 23 to 26.

41. A vaccine comprising a bacterium according to claim 40 and a pharmaceutically acceptable carrier or diluent.

42. A bacterium according to claim 40 for use in a method of vaccinating a human or animal.

43. Use of a bacterium according to claim 40 for the manufacture of a medicament for vaccinating a human or animal.

44. A method for raising an immune response in a mammalian host, which method comprises the step of administering to the host a bacterium according to claim 40.

45. A method for preparing an attenuated bacterium, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to any one of claims 23to 26; and

(b) introducing a non-reverting mutation into a thus-identified conditional essential gene of the bacterium, thereby to attenuate the bacterium.

46. A method for the preparation of a vaccine, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to any one of claims 23 to 26;

(b) introducing a non-reverting mutation into a thus-identified conditional essential gene of the bacterium, thereby to attenuate the bacterium; and

(c) formulating the attenuated bacterium with a pharmaceutically acceptable carrier or diluent.

47. A method for raising an immune response in a mammalian host, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to any one of claims 23 to 26;

(b) introducing a non-reverting mutation into a thus-identified conditional essential gene of the bacterium, thereby to attenuate the bacterium;

(c) formulating the attenuated bacterium with a pharmaceutically acceptable carrier or diluent; and

(d) administering to the host the attenuated bacterium thus formulated.